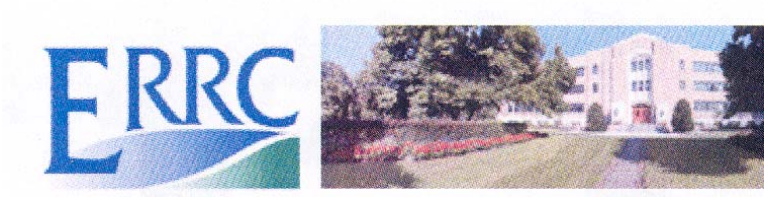

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Title: Preliminary Results of Radio Pasteurization (RAP)

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Preliminary Results of Radio Pasteurization (RAP)

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Abstract. *Radio frequency energy was investigated as a nonthermal alternative to thermal pasteurization. Two RF power supply systems were assembled and provided frequencies in the range of 20 kHz to 27 MHz. Electric field strengths of 14 to 30 kV/cm were applied to suspensions of Saccharomyces cerevisiae in water over a temperature range of 28 to 55°C. The flow rate was 1.2 l/min and the number of exposures to the fields ranged from 1 to 30. The population of S. cerevisiae was reduced by >5 log following 30 exposures to a 100 kHz, 25 kV/cm field at 28°C. Increasing the field strength and temperature, as well as decreasing the frequency enhanced inactivation. These preliminary results of radio pasteurization, RAP, are encouraging and will be used in an effort to extend this technique to bacteria in vegetable and fruit juices.*

Keywords. Food safety, nonthermal pasteurization, RF energy, yeasts, electric field

Introduction

Consumers are drinking more vegetable and fruit juices than ever before. In addition, they are demanding that these beverages have fresh like qualities and nutrient content. In order to assure the safety of these products, pasteurization is required. Conventional thermal pasteurization can affect sensorial qualities. Therefore, alternate pasteurization processes are actively being sought. Examples include the use of high-pressure and electromagnetic energy. The use of radio frequency (RF) electromagnetic energy has been studied for over 50 years as a pasteurization method. Although reports often claimed pasteurization had been achieved at nonthermal conditions, these claims could never be confirmed. The cause of these unsubstantiated claims is most likely due to incorrect temperature measurements (Geveke et al. in press). In order to test these claims, Brunkhorst et al. (2000) assembled a RF system that accurately controlled temperature. When an electric field strength of 0.5 kV/cm at a frequency of 18 MHz was applied to apple cider, beer, deionized water, and tomato juice, nonthermal effects on *Escherichia coli* K-12, *Listeria innocua*, and yeast were not observed (Geveke et al. in press).

Sale and Hamilton (1967) applied square wave DC pulses to suspensions of vegetative bacteria and yeast cells and concluded that a minimum electric field strength of 5 kV/cm is necessary to achieve inactivation (Figure 1). They also observed that increasing the electric field strength increased inactivation. Zimmermann et al. (1974) proposed the dielectric rupture theory to explain the inactivation. When an external electric field is applied to a cell in a suspension, an induced voltage is formed across the membrane due to its capacitance. As the voltage is increased, the opposite charges on either side of the membrane are attracted to each other with greater force and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures. There is a lag between the applied voltage and the induced voltage across the membrane due to its capacitance. Kotnik et al. (1998) reported on the time course of transmembrane voltage induced by RF fields. Although they stated that it is generally very difficult to predict the peak value of the induced transmembrane voltage, it is clear from their analysis that the voltage is significantly reduced as the frequency is increased from 100 kHz to 1 MHz.

Geveke et al. (in press) hypothesized that nonthermal pasteurization might be achieved if RF energy with a field strength of ≥5 kV/cm were applied. They also hypothesized that if the frequency were reduced to <18 MHz, nonthermal pasteurization might likewise be achieved. The objective of this work was to assemble RF systems that would be capable of applying a field strength of >10 kV/cm over a frequency range of 10 kHz to 27 MHz. An additional objective was to determine if nonthermal radio pasteurization, RAP, was feasible using these systems.

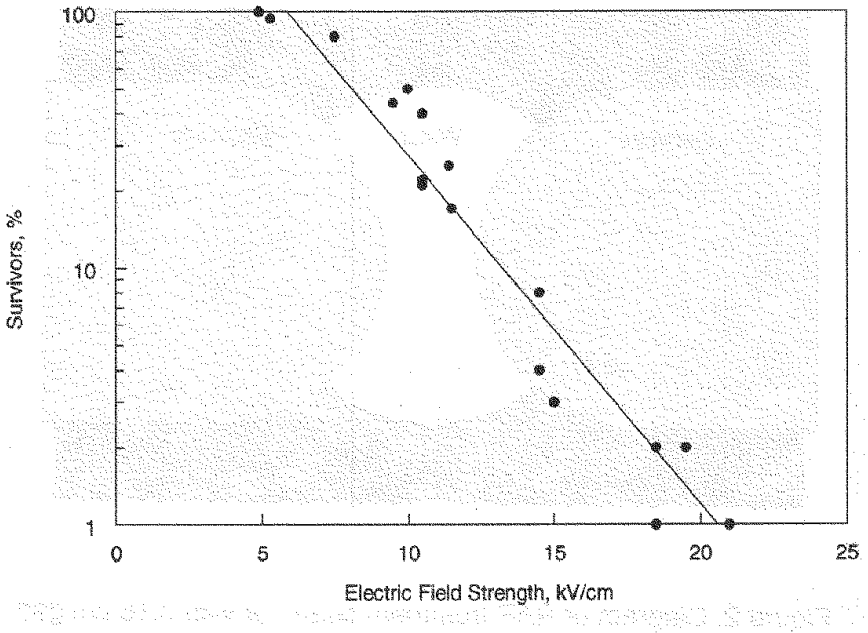

Figure 1. Inactivation of *E. coli* by 10 square wave DC pulses at room temperature (Sale and Hamilton, 1967).

Materials and Methods

Equipment

Two RF power supplies were used in this study. The first power supply included a fixed frequency 27 MHz generator described previously (Brunkhorst et al., 2000). In order to increase the field strength from 0.5 kV/cm to > 10 kV/cm, a new treatment chamber was designed and built. The chamber was made of Teflon. Liquid flowed through a bore with a diameter of 0.64 cm. Two stainless-steel electrodes were inserted into the Teflon perpendicular to the liquid flow. The electrodes were cylindrical, with a 0.64 cm diameter, and their ends were rounded and polished. At their closest proximity the electrodes were 0.16 cm apart as shown in Figure 2. The output of the RF generator was connected to the electrodes. The peak voltage generated by the 27 MHz power supply was 2.2 kV.

A schematic of the experimental system is shown in Figure 3. It includes a stainless steel feed tank. A peristaltic pump (Cole-Parmer, Vernon Hills, IL; driver model 7523-40; head model 77200-62) supplied the feed to the RF system at flow rates of 1.2 l/min through Norprene pump tubing (Cole-Parmer, model 06402-15). The inlet temperature to the RF treatment chamber was controlled using a stainless-steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) and a temperature controller (Cole-Parmer, model CALL 9400)

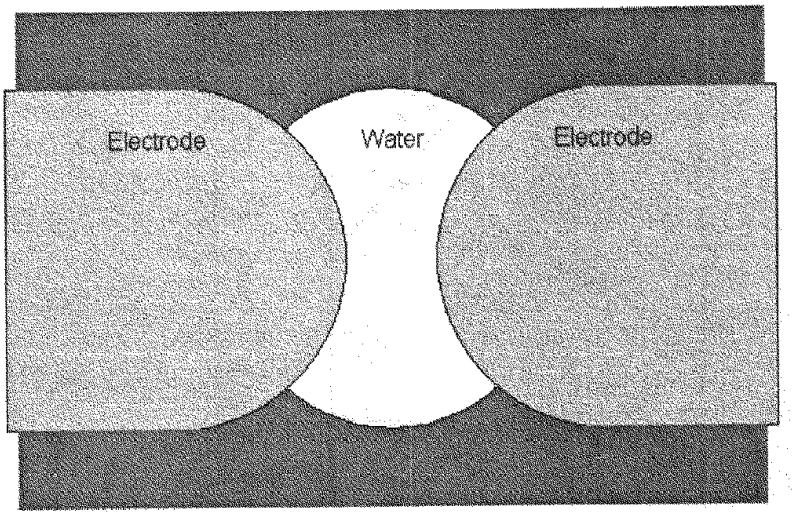

Figure 2. Diagram of RAP treatment chamber with 0.16 cm gap

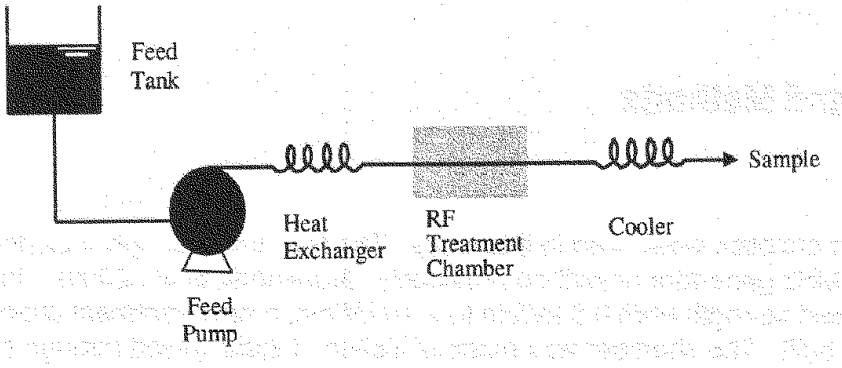

Figure 3. Schematic Diagram of RAP System

The temperatures of the process fluid immediately before and after the RF treatment chamber were measured with fiber optic sensors (Luxtron, Santa Clara, CA, model 950). The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0).

The process fluid was quickly cooled after exiting the treatment chamber to < 25°C using a stainless-steel cooling coil submerged in a water bath. The length of time for the fluid to travel from the treatment chamber to the outlet of the cooler was less than four seconds. In some cases, the effect of exposure to multiple treatments was desired, and fluid was recycled.

In order to study RF inactivation of microorganisms at lower frequencies, a second RF power supply system was assembled. It consisted of four 1 kW amplifiers (Industrial Test Products, Port Washington, NY, model 1000A) and four output transformers (Industrial Test Products). These were connected in series and produced a peak voltage of > 4.0 kV over a frequency range of 20 to 100 kHz. A function generator (Tektronix, Beaverton, OR; model AFG 310) drove the amplifiers. The voltage and current were measured with an oscilloscope (Tektronix, model TDS210) and a voltage divider (Ross Engineering, Campbell, CA; model VD15-8.3-A-KB-A).

Culture

Saccharomyces cerevisiae (ATCC 16664) was purchased from American Type Culture Collection (Manassas, VA). The yeast was cultured in yeast malt broth at 28°C for 24 hours. The stationary phase culture was diluted with sterilized deionized water to yield an approximately 6 log cfu/ml population. The solution's pH was 7.0 and its conductivity was 60 µS/cm.

Sampling and Analysis

Duplicate samples were taken periodically of the feed and product. Appropriate dilutions of the samples were plated on tryptose agar using a spiral plater (Spiral Biotech, Bethesda, MD; model Autoplate 4000) and incubated at 37°C for 48 h. Enumerations were made with a colony counter (Spiral Biotech, model 500A).

Results and Discussion

Radio frequency (RF) energy successfully inactivated *Saccharomyces cerevisiae* at nonthermal conditions. The extent of microbial inactivation is dependent on the electric field strength, number of exposures, frequency, and temperature.

The population of *S. cerevisiae* remained constant after being exposed to ten treatments with ar electric field strength of 14 kV/cm and a frequency of 27 MHz at a temperature of 28°C. The electric field strength required to achieve inactivation is generally considered to be approximately 5 kV/cm (Sale and Hamilton, 1967; Hulsheger et al., 1981). The reason for the lack of inactivation may be due to the high frequency used in our study. At a frequency of 27 MHz, the period is 37 ns. As explained in the introduction section, because the cell membrane has a capacitance, the induced field lags behind the applied field (Kotnik et al., 1998). If the lag time was greater than 37 ns, the induced field may have been less than 14 kV/cm.

A series of experiments were performed using the low-frequency RF system. At a frequency of 100 kHz and a temperature of 28°C, nonthermal radio pasteurization was obtained. The concentration of *S. cerevisiae* was reduced by 5.6 log after being exposed 30 times to a 25 kV/cm electric field strength as shown in Figure 4. A 3.2 log reduction was achieved after 10 exposures. These nonthermal inactivation results are believed to be due to dielectric rupture of the cells (Zimmermann et al., 1974).

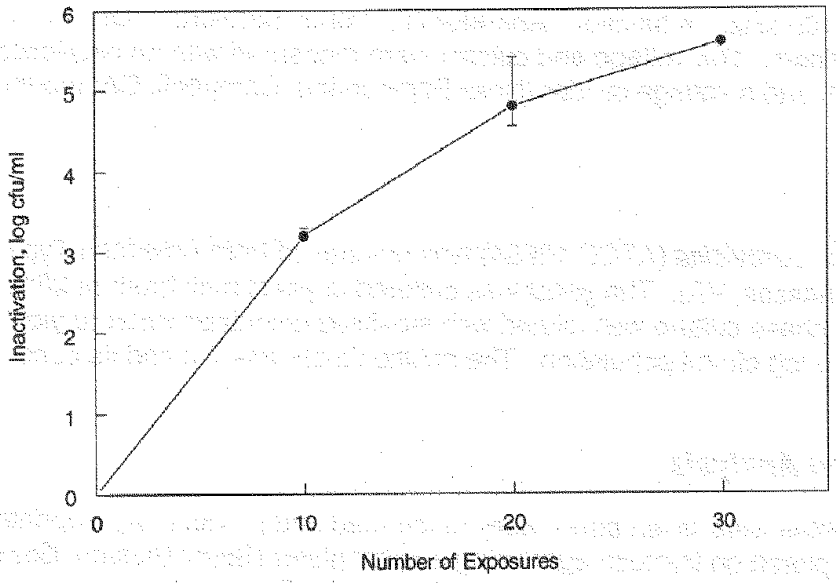

Figure 4.inactivation of Saccharomyces cerevisiae at 28°C using RAP technology. An electric field strength of 25 kV/cm at a frequency of 100 kHz was applied. Error bars indicate standard deviations.

Experiments were conducted over the frequency range of 20 to 80 kHz. A single exposure of 25 kV/cm electric field strength at a temperature of 45°C was applied to *S. cerevisiae*. The greatest inactivation occurred at the lowest frequencies as shown in Figure 5. Extrapolating these results to much higher frequencies indicates that *S. cerevisiae* would not be inactivated at 27 MHz. Kotnik et al. (1998) theorized that as frequency is increased up to the MHz region, the induced field strength across the cell membrane is substantially reduced. A reduced field strength would minimize or prevent inactivation. Our results agree with the analysis of Kotnik et al.

In light of the above results, the remaining experiments were conducted at a frequency of 20 kHz. The concentration of *S. cerevisiae* was reduced by over 4.6 log by a single exposure to a 30 kV/cm field strength at a temperature of 55°C as shown in Figure 6. Inactivation was greater using a field strength of 30 kV/cm rather than 20 kV/cm for outlet temperatures of 40 to 55°C. This was true even though the inlet temperatures to the treatment chamber were higher in the case of the 20 kV/cm treatments. These results prove that the electric field strength plays an important role in inactivation in addition to temperature.

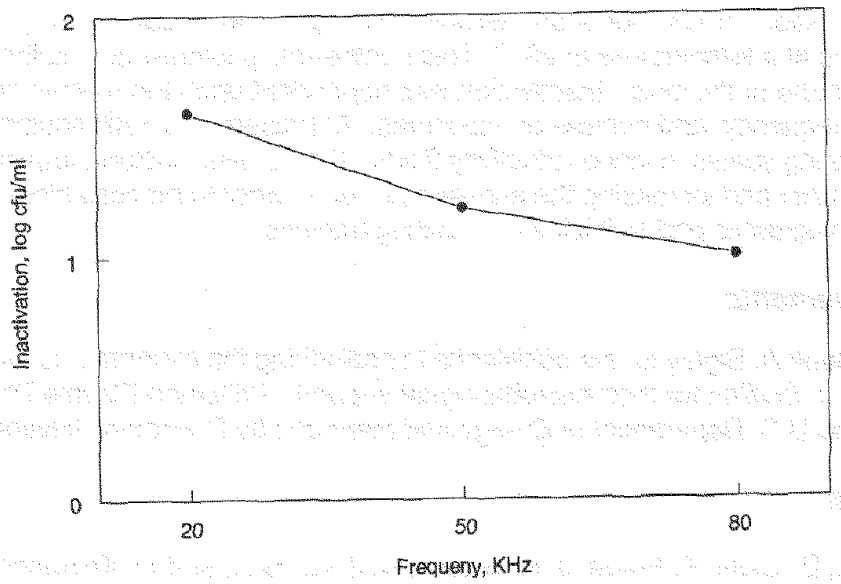

Figure 5. Inactivation of *S. cerevisiae* at 45°C and an electric field strength of 25 kV/cm.

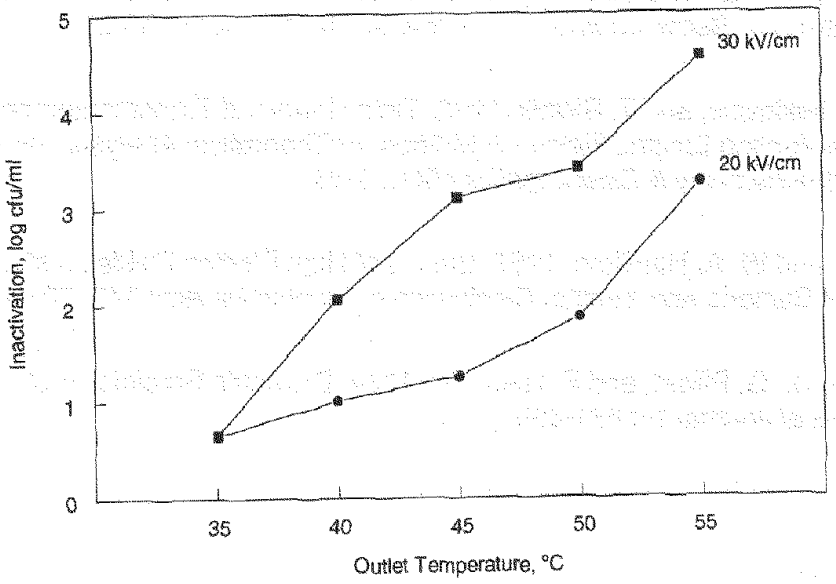

Figure 6. Inactivation of *S. cerevisiae* at a frequency of 20 kHz.

Conclusion

Radio pasteurization, or RAP for short, reduced the population of *Saccharomyces cerevisiae* in water by 5.6 log at a temperature of 28°C. This nonthermal pasteurization is thought to be due to dielectric rupture of the cells. Inactivation was dependent upon the electric field strength, temperature, frequency, and number of exposures. At present, the RAP equipment is only capable of treating yeasts in low conductivity fluids. Future plans include redesigning the treatment chamber and increasing the supplied power in order to process higher conductivity fluids such as vegetable and fruit juices containing bacteria.

Acknowledgements

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